

the parent alpha-amylase;

D1 (b) identifying in the [modeled] three-dimensional structure depicted in Appendix 1 [obtained in step(a)] at least one structural part [of the parent] wherein an alteration in [said] a corresponding structural part in said parent alpha-amylase is predicted to result in an altered property, wherein said altered property is selected from the group consisting of substrate specificity, substrate binding, substrate cleavage pattern, temperature stability, pH dependence of enzymatic activity, pH dependence of stability, stability towards oxidation,  $Ca^{2+}$ -dependency and specific activity;

[(c)] (b) modifying the sequence of a nucleic acid encoding the parent alpha-amylase to produce a nucleic acid encoding a deletion, insertion, or substitution of one or more amino acids at a position corresponding to said structural part; and

[(d)] (c) expressing the modified nucleic acid in a host cell to produce the variant alpha-amylase, wherein the variant has alpha-amylase enzymatic activity and has at least one altered property relative to the parent.

Add new claims 76-78 reading as follows:

--76. A method of producing a variant of a parent alpha-amylase having an altered property relative to the parent, wherein the parent alpha-amylase has the sequence of SEQ ID Nos: 2, 4, 6, or 13, or has a sequence at least 70% homologous to the sequence of SEQ ID Nos: 2, 4, 6, or 13 when homology is determined by the GAP program (Genetic Computer Group, Version 7.0) using default values for GAP penalties, said method comprising

D2 (a) providing a three-dimensional alpha-amylase structure that exhibits A, B, and C domains and three metal ions at the junction of the A and B domains;

(b) identifying in said three-dimensional structure at least one structural part wherein an alteration in the structural part or a corresponding structural part in said parent alpha-amylase is predicted to result in an altered property and wherein said altered property is selected from the group consisting of substrate specificity, substrate binding, substrate cleavage pattern, temperature stability, pH dependence of

enzymatic activity, pH dependence of stability, stability towards oxidation,  $\text{Ca}^{2+}$ -dependency and specific activity;

(c) modifying the sequence of a nucleic acid encoding the parent alpha-amylase to produce a nucleic acid encoding a deletion, insertion, or substitution of one or more amino acids at a position corresponding to said structural part; and

(d) expressing the modified nucleic acid in a host cell to produce the variant alpha-amylase,

wherein the variant has alpha-amylase enzymatic activity and has at least one altered property relative to the parent.--

--77. A method of producing a variant of a parent alpha-amylase having an altered property relative to the parent, wherein the parent alpha-amylase has the sequence of SEQ ID Nos: 2, 4, 6, or 13, or has a sequence at least 70% homologous to the sequence of SEQ ID Nos: 2, 4, 6, or 13 when homology is determined by the GAP program (Genetic Computer Group, Version 7.0) using default values for GAP penalties, said method comprising

(a) identifying in a three-dimensional alpha-amylase structure at least one structural part wherein an alteration in the structural part or in a corresponding structural part in said parent alpha-amylase is predicted to result in an altered property, and wherein:

(i) said three-dimensional structure exhibits A, B, and C domains and three metal ions at the junction of the A and B domains;

(ii) said structural part comprises part of said B domain, wherein said B domain corresponds to residues 104-205 in the structure depicted in Appendix 1; and

(iii) said altered property is selected from the group consisting of substrate specificity, substrate binding, substrate cleavage pattern, temperature stability, pH dependence of enzymatic activity, pH dependence of stability, stability towards oxidation,  $\text{Ca}^{2+}$ -dependency and specific activity;

(b) modifying the sequence of a nucleic acid encoding the parent alpha-amylase to produce a nucleic acid encoding a deletion, insertion, or substitution of one

or more amino acids at a position corresponding to said structural part; and  
(c) expressing the modified nucleic acid in a host cell to produce the variant alpha-amylase.  
wherein the variant has alpha-amylase enzymatic activity and has at least one altered property relative to the parent.--

--78. A method of producing a variant of a parent alpha-amylase having an altered property relative to the parent, wherein the parent alpha-amylase has the sequence of SEQ ID Nos: 2, 4, 6, or 13, or has a sequence at least 70% homologous to the sequence of SEQ ID Nos: 2, 4, 6, or 13 when homology is determined by the GAP program (Genetic Computer Group, Version 7.0) using default values for GAP penalties, said method comprising

(a) identifying in a three-dimensional alpha-amylase structure at least one structural part wherein an alteration in the structural part or in a corresponding structural part in said parent alpha-amylase is predicted to result in an altered property, and wherein:

(i) said three-dimensional structure exhibits A, B, and C domains and three metal ions at the junction of the A and B domains;

(ii) said structural part comprises part of a substrate-binding region, wherein said region comprises residues corresponding to one or more of residues Trp13, Tyr14, Asn17, Asp18, Ser50, Gln51, Ala52, Asp53, Val54, Gly55, Tyr56, Lys70, Arg74, Lys76, Val102, His105, Gly107, Gly108, Ala109, Trp138, Thr163, Asp164, Trp165, Asn172, Glu189, Tyr193, Leu196, Met197, Tyr198, Ala199, Arg229, Asp231, Ala232, Lys234, His235, Glu261, Trp263, His327, Asp328, Gln333, Ser334, and Leu335 of the structure depicted in Appendix 1; and

(iii) said altered property is selected from the group consisting of substrate specificity, substrate binding, substrate cleavage pattern, temperature stability, pH dependence of enzymatic activity, pH dependence of stability, stability towards oxidation,  $\text{Ca}^{2+}$ -dependency and specific activity;

(b) modifying the sequence of a nucleic acid encoding the parent alpha-amylase to produce a nucleic acid encoding a deletion, insertion, or substitution of one or more amino acids at a position corresponding to said structural part; and